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The Investigation of Phytopathogenic Bacteria by Serological and Biochemical Methods

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THE INVESTIGATION OF PHYTOPATHOGENIC BACTERIA BY SEROLOGICAL AND BIOCHEMICAL METHODS.

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From the National Collection of Type Cultures, Medical Research Council, Lister Institute, London.

Although an extensive literature has developed, for the most part in the United States, with regard to the morphology and pathogenicity of various organisms isolated from phytopathogenic lesions, references to serological investigations in connection with pathogenic plant bacteria are scanty in the extreme. C. O. Jensen (1918) has investigated the serological relations of different strains of the crown gall organism, *B. tumefaciens*, and found that a danish strain of this organism, derived from a spontaneous tumour in a species of *Chrysanthemum*, agreed with an american strain of the same organism (isolated from *Chrysanthemum frutescens*) culturally and morphologically but deviated from it serologically in that it was not affected by an agglutinating serum prepared by intravenous injection of rabbits with the american strain. Conversely the american strain was not agglutinated by serum derived from the danish strain. Doidge (1917) working with *B. citrimaculans* found that the serum of a rabbit inoculated with this organism agglutinated the homologous strain at dilutions up to 1/2000 while Paine and Lacey (1923) showed that the two organisms, *B. lathyri* and *B. phaseoli*, although possessing distinctive cultural characters gave evidence of cross agglutination on serological investigation.

A considerable number of cultures of bacteria associated with diseases of plants having been received by the National Collection of Type Cultures through the courtesy of Dr Erwin F. Smith of the United States Department of Agriculture, a favourable opportunity presented itself for examining this material by cultural, biochemical and serological methods along with other strains of plant bacteria, which had been received from time to time from various sources.

Of the thirty-three strains received from Dr Erwin Smith, eleven produced greenish fluorescent pigment, ten formed yellow pigment while the remainder were colourless. The striking resemblance of the first group to the well-known *B. fluorescens liquefaciens*, Flügge, and *B. fluorescens non-liquefaciens*, Lehm. and Neum., made the combined investigation of these organisms desirable.

1. Fluorescent group.

The fluorescent organisms received from Dr Erwin Smith were labelled as follows: *B. delphinii B. lachrymans B. angulatum B. tabacum B. mori B. coronafaciens B. syringæ B. aptatum B. viridili-*

vidum B. marginale and *B. atrofaciens.* For comparison were available a strain of *B. mori* isolated by Dr H. Wormald, S.E. Agricultural College, Wye, and strains of *B. barkeri* and *B. tolaasi* received from Dr S. G. Paine, Imperial College of Science, South Kensington, in addition to four strains of *B. fluorescens* collected from various sources.

Fermentation reactions.

With the exception of *B. syringæ*, which produced acid and gas in lactose glucose mannite and saccharose, all the strains examined agreed closely in producing acid in glucose and having no action on lactose mannite dulcite and saccharose. With *B. marginale* acidity was not observed until the tenth day, while *B. coronafaciens* produced slight acid in saccharose and one of the strains of *B. fluorescens* (no. 1382) gave acid in mannite and saccharose. A large number of the strains were actively motile but no motility was observed with one of the strains of *B. mori* or with *B. lachrymans B. tabacum B. coronafaciens* and *B. syringæ.* Gelatin was liquefied by all the strains, with the exception of *B. mori* and *B. fluorescens non-liquefaciens* (no. 912). In litmus milk strong alkalinity was shown by all the strains examined, with the exception of *B. syringæ B. fluorescens liquefaciens* (no. 964) and *B. delphinii,* which gave permanent acid reactions, and *B. fluorescens* (no. 950) which produced an orange coloration and peptonised the medium in a similar manner to that observed subsequently with strains of *B. pyocyaneus.* On subculture from milk and on growth on Emrys-Roberts' medium (1914) the production of pyocyanin definitely established this organism's correct relationships.

Serological data.

A large number of agglutinating sera were prepared from these strains by means of intravenous inoculation of rabbits and the results obtained with agglutination experiments show the significant relationship that exists between *B. fluorescens liquefaciens* and *non-liquefaciens* on the one hand and the fluorescent plant organisms on the other. The following table clearly shows this relation. The titre of the various sera in this group and in those to be considered subsequently was of the order of 1/8000, agglutinations being carried out provisionally at a dilution of 1/100. It is proposed to make the complete investigation of the agglutination and absorption relationships of these organisms the subject of a further communication when recorded data on this aspect will be discussed.

The close serological relationship between *B. delphinii B. lachymans B. angulatum B. tabacum B. mori* and *B. viridilividum* is clearly evident. *B. aptatum B. atrofaciens* and *B. coronafaciens* have several important relations in common, *B. aptatum* and *B. atrofaciens* being agglutinated by *fluorescens non-liquefaciens* (912) serum and *B. aptatum* and *B.*

TABLE I.

Name of organism.	Agglutinable at 1/100 dilutions of high titre serum of:—															
	B. fluorescens liq. (950).	*B. fluorescens liq.* (64).	*B. fluorescens non-liq.* (912).	*B. delphinii.*	*B. lachrymans.*	*B. angulatum.*	*B tabacum.*	*B. mori* (E.F.S.).	*B. coronafaciens.*	*B. syringæ.*	*B. aptatum.*	*B. viridilividum.*	*B. marginale.*	*B. atrofaciens.*	*B. tolaasi.*	*B. barkeri.*
B. fluorescens liq. (950).	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
,, (1382)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
,, (964)	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
,, *non-liq.* (912)	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−
B. delphinii	−	−	−	+	−	−	+	+	+	−	−	−	−	−	−	−
B. lachrymans	−	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−
B. angulatum	−	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−
B. tabacum	−	−	−	+	+	+	+	−	−	−	+	+	−	−	−	−
B. mori (E.F.S.)	−	−	−	+	+	+	+	+	+	−	−	+	−	−	−	−
,, (Wormald)	−	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−
B. coronafaciens	−	+	−	+	−	+	+	−	+	−	+	+	−	−	−	−
B. syringæ	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
B. aptatum	−	+	+	−	−	+	+	+	+	−	+	+	−	+	−	−
B. viridilividum	−	−	−	+	+	+	+	−	+	−	−	+	−	−	−	−
B. marginale (E.F.S.)	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
B. atrofaciens	−	−	+	−	−	+	+	−	+	+	−	−	−	+	−	−
B. tolaasi	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+
B. barkeri	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+

+ = positive. − = negative.

coronafaciens by *B. fluorescens liquefaciens* serum (964). *B. tolaasi* and *B. barkeri* have close serological affinities with each other, the former also showing cross agglutination with *B. fluorescens non-liquefaciens* (no. 912). *B. syringæ* stands by itself, except for the agglutination of *B. atrofaciens* by *B. syringæ* serum. *B. marginale* (E.F.S.) has no relationship with any of the other fluorescent organisms.

Recently a strain *B. marginale* (N.A.B.), which was received some years ago from Miss N. A. Brown of Dr Erwin Smith's Department was sent to the National Collection by Miss Mehta, working at the Department of Botany, Imperial College of Science, South Kensington (1924). This strain differs greatly from that received from Dr Erwin Smith, producing pyocyanin as well as fluorescin in culture. A comparison of the serological relations of both these strains with eleven strains of *B. pyocyaneus* (Flügge), collected from various sources, is appended. Agglutination tests were also carried out with the entire series of fluorescent organisms against the various *B. pyocyaneus* sera mentioned in the table. In every case the results obtained were negative.

TABLE II.

Name of organism.	Agglutinable at 1/100 dilution of high titre serum of—						
	B. pyocyaneus (kangaroo).	*B. pyocyaneus* (goat).	*B. pyocyaneus* (Fildes II.).	*B. pyocyaneus* (Panda).	*B. pyocyaneus* (Cobbett).	*B. marginale* (N.A.B.).	*B. marginale* (E.F.S.).
B. pyocyaneus (kangaroo) . .	+	+	+	+	−	−	−
,, ,, (goat) . . .	+	+	−	−	−	−	−
,, ,, (Fildes II.) . .	−	−	+	+	−	−	−
,, ,, (Panda) . . .	−	−	+	+	−	+	−
,, ,, (Cobbett) . .	−	−	+	+	+	−	−
,, ,, (monkey) . .	−	−	+	+	−	−	−
,, ,, (Fildes III.) . .	+	−	+	+	−	−	−
,, ,, (Eyre I.) . .	−	−	−	−	−	−	−
,, ,, (Eyre II.) . .	−	−	−	−	−	−	−
,, ,, (Dudgeon) . .	−	−	+	+	−	−	−
,, ,, (Mehta) . . .	−	−	−	+	−	+	−
B. marginale (N.A.B.). . .	−	−	−	−	−	+	−
,, ,, (E.F.S.) . . .	−	−	−	−	−	−	+

+ = positive. − = negative.

It will be seen that Dr Erwin Smith's *B. marginale* has no serological affinity with any of the strains of *B. pyocyaneus* or with Miss N. A. Brown's strain of *B. marginale*. A serum prepared from the latter organism agglutinates *B. pyocyaneus* (Panda) and *B. pyocyaneus* (Mehta) only and is not agglutinated by any of the other test sera. *B. pyocyaneus* (Mehta) purports to be a descendant of *B. pyocyaneus* (Cobbett) which was sent to the Imperial College of Science some time ago but which has, apparently, lost relationship with the original strain and has taken on entirely new serological characters. This point requires further elucidation. The cultural characters of all the strains of *B. pyocyaneus* and of *B. marginale* (N.A.B.) are very similar. They all produce acid rapidly in glucose peptone water but have no action on lactose mannite dulcite or saccharose. *Litmus milk* becomes orange in colour and subsequently peptonised. Erwin Smith's strain of *B. marginale* produces acid and clot in milk and acidifies glucose very slowly.

2. Yellow group.

Eleven organisms producing yellow pigment were received from Dr Erwin Smith, described as *B. campestre B. malvacearum B. michiganense B. pruni B. phaseoli B. phaseoli var. sojense B. gummisudans B. stewarti B. pelargoni B. vitians* and *B. tracheiphilus.* For purposes of comparison were obtained three strains of *B. campestre* from the Agricultural College of Ontario, Guelph, Dr S. Paine and the New York Agricultural Experimental Station respectively, a strain of *B. malvacearum* from Prof. Ashby, Trinidad, and strains of *B. hyacinthi* and *B. lathyri* from Dr Paine.

Fermentation reactions.

Two of the strains of *B. campestre* (E.F.S. and Paine) gave late acid in lactose as did also *B. phaseoli var. sojense.* All the strains of *B. campestre* and also *B. lathyri* gave slight acid in glucose. *B. campestre* (Paine) gave in addition gas in glucose and saccharose. *B. stewarti* gave acid in lactose glucose mannite and saccharose, while *B. tracheiphilus* gave no growth in carbohydrate media. The other organisms did not alter the reaction of the test sugar media. All the organisms with the exception of *B. stewarti* and *B. hyacinthi* liquefy gelatin in from one day to three weeks.

Serological data.

The following table gives the results of the serological investigations so far carried out:—

TABLE III.

Name of organism.	Agglutinable to 1/100 dilution of high titre serum of—									
	B. campestre (Guelph).	*B. campestre* (E.F.S.).	*B. campestre* (Paine).	*B. malvacearum* (E.F.S.).	*B. malvacearum* (Trinidad).	*B. pruni.*	*B. gummisudans.*	*B. phaseoli.*	*B. phaseoli var. sojense.*	*B. pelargoni.*
B. campestre (Guelph)	+	−	−	−	−	−	−	−	−	−
,, ,, (E.F.S.)	+	+	−	+	+	−	+	+	+	+
,, ,, (Paine)	−	−	+	−	−	−	−	−	−	−
,, ,, (New York)	+	+	−	+	+	−	−	+	−	−
B. malvacearum (E.F.S.)	+	+	−	+	+	−	−	+	+	+
,, ,, (Trinidad)	+	+	−	+	+	−	−	+	+	+
B. michiganense	−	−	−	−	−	−	−	−	−	−
B. pruni	−	−	−	−	−	+	−	−	−	−
B. gummisudans	−	−	−	−	+	−	+	+	−	−
B. phaseoli	+	+	−	+	+	−	+	+	+	+
B. phaseoli var. sojense	−	−	−	+	+	−	−	−	+	+
B. stewarti	−	−	−	−	−	−	−	−	−	−
B. pelargoni	−	+	−	+	+	−	−	+	−	+
B. vitians	−	+	−	+	+	−	+	−	+	+
B. tracheiphilus	−	−	−	−	−	−	−	−	−	−
B. hyacinthi	−	−	−	−	−	−	−	−	−	−
B. lathyri	−	−	−	−	−	−	−	−	−	−

\+ = positive. − = negative

It will be observed that two strains of *B. campestre* (E.F.S. and New York) agree very closely and are both agglutinated by *B. campestre* (Guelph) serum. The strain of this organism isolated by Dr Paine stands by itself with regard to all the organisms examined in this group. The serological relations of both the strains of *B. malvacearum* (E.F.S. and Trinidad) are practically identical and it is manifest that the closest relationship exists between *B. campestre* (certain strains) *B. malvacearum B. phaseoli* and its variety *sojense B. pelargoni* and *B. vitians*. With regard to the three organisms first mentioned, this result confirms Dr Erwin Smith's opinion regarding their closely related kinship (1920). So far as at present investigated *B. michiganense B. pruni B. stewarti B. tracheiphilus B. hyacinthi* and *B. lathyri* have no relationship to each other or to other members of the group.

3. White group.

As a matter of convenience the remaining organisms received from Dr Erwin Smith were provisionally placed in a residual group though it was not anticipated that there was necessarily any close connection between the different species. They comprised *B. cannæ B. amylovorus B. trifoliorum B. panici B. carotovorus B. tumefaciens* (Peach) *B. beticolum B. aroideæ* (two strains) and *B. phytophthorus*. For comparison were available strains of *B. atrosepticus B. amylovorus* and *B. carotovorus* from Guelph, *B. carotovorus* from Dr Wormald, *B. carotovorus* and *B. phytophthorus* through Miss Lacey, Imperial College of Science and *B. tumefaciens* (hop and michaelmas daisy) originally obtained from Dr Erwin Smith himself. Strains of *B. solanisaprus* and of *B. proteamaculans* were also received from Dr Paine.

Fermentation reactions.

Action on carbohydrates.—The strains of *B. carotovorus* (with the exception of the Guelph strain) *B. phytophthorus* and *B. solanisaprus* gave identical sugar reactions, fermenting lactose glucose mannite and saccharose with production of gas. Acid production with clot was observed in litmus milk, gelatin was rapidly liquefied and vigorous

TABLE IV.

Name of organism.	Agglutinable at 1/100 dilutions of high titre serum of—			
	B. carotovorus (E.F.S.).	*B. carotovorus* (Bewley).	*B. phytophthorus* (Appel).	*B. solanisaprus.*
B. carotovorus (E.F.S.)	+	−	−	−
,, (Bewley)	−	+	−	−
,, (turnip)	−	−	−	−
,, (Guelph)	−	−	−	−
B. phytophthorus (E.F.S.)	−	+	−	−
,, (Appel)	−	+	+	−
B. solanisaprus	+	−	−	+

motility was evident in broth cultures. There is a distinct serological relationship between the three organisms as is shown in table IV.

Of the other organisms *B. aroideæ* produced acid but no gas in lactose glucose mannite and saccharose, *B. beticolum* acid and gas in glucose and saccharose and *B. amylovorus* acid in glucose and saccharose *B. cannæ, B. trifoliorum* and *B. panici* did not alter the reaction of any of the test sugars employed. The strain of *B. carotovorus* received from Guelph differed from the other strains and produced acid only in glucose. Likewise the strain of *B. amylovorus* from Guelph differed from the Erwin Smith type, producing acid but no gas in glucose and mannite only and not liquefying gelatin. The three strains of *B. tumefaciens* agreed fairly closely, but the michaelmas daisy strain gave acid in glucose and saccharose while the other two strains had no effect on sugar media: they were all non-motile and did not liquefy gelatin. These reactions are identical with those shown by *B. atrosepicus* (Guelph), a serum prepared from which agglutinated *B. tumefaciens* (hop) to quarter titre.

Summary.

An extensive series of bacteria associated with diseases of plants has been examined by cultural biochemical and serological methods.

Cultural appearances on agar enabled a preliminary grouping into (1) fluorescent (2) yellow and (3) white organisms, the biochemical reactions within the first two groups proving fairly consistent.

So far as the serological examination of strains within the groups has proceeded it has revealed important groupings, the further analysis of which will be the subject of a later communication.

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